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THE PLASMOEBA OF DENGUE : A BRIEF DESCRIPTION OF THE EARLIEST PHASES OF ITS PLASMIC CHARACTERISTICS

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tively, twenty-two and twenty-four years previously, and both patients had followed a protracted mercurial treatment. Five injections of antisyphilitic serum (10 c.c.) were administered a week apart to each one of the patients, but no change could be noted. Still, another case might be classed under this heading that did prove more encouraging:

Mr. C., broker, aged fifty-nine years, contracted syphilis twenty-five years ago. About 1897, four ulcers, presumably trophic, appeared, two upon the sole of each foot, and, in spite of all internal and external treatment, persisted for years. Over a year ago, symptoms of ataxia began to manifest themselves. Loss of reflex was first noticed, and this was soon followed by a few lightning pains and slight incoordination.

On December 30, 1903, treatment was begun by an injection of 10 c.c. of the antisyphilitic serum.

January 3, 1904. The patient fell and broke his leg, which accident confined him to his bed for four weeks, and to the house for two weeks longer. During all this time, injections of the serum were regularly made every seven days. At the end of four weeks the ulcers had completely healed, and the pains had subsided.

For six months this patient has been up and attending to his usual business; there has been no recurrence of pains, no disposition toward a breaking down of the skin of the feet preceding a reformation of ulcers, and, what is still more remarkable, there seems to be a slight return of patellar reflex.

Many circumstances in this case have contributed to the favorable issue. First, the ataxia was in its incipiency; second, the patient's recumbent position in bed, caused by his accident; lastly, his excellent health and good habits were important factors that must not be ignored in the account of the case.

500 Fifth Avenue.

Ladies' Auxiliary for Free Hospital for Poor Consumptives Formed.—On December 8th, an auxiliary of the Free Hospital for Poor Consumptives was organized by about fifty prominent club women. The object of the auxiliary is at present to assist in collecting funds for three new buildings; one at White Haven for cases not incipient, but yet not hopeless; a second in the nature of a farm for convalescents; and a third in Philadelphia for moribund cases. Dr. Lawrence F. Flick made an address. The following officers were elected: Chairman, Mrs. William M. Kerr; first vice-chairman, Mrs. Talcot Williams; second vice-chairman, Mrs. M. Ravenel; secretary, Mrs. C. J. Hatfield; treasurer, Mrs. William M. Lybrand. Among those who joined the auxiliary were Mrs. J. Sparhawk Jones, Miss Tucker, Mrs. James Speer, Mrs. Elliot, Miss Benson, Miss Muir, Miss Ellis, Miss Leidy, Miss Keating, Mrs. Austin, Miss Austin, Mrs. C. E. Morris, Mrs. M. M. Arnold, Mrs. C. A. Steele, Mrs. Fetterolf, Mrs. C. Y. White, and Mrs. D. Edsall.

THE PLASMŒBA OF DENGUE; A BRIEF DESCRIPTION OF THE EARLIEST PHASES OF ITS PLASMIC CHARACTERISTICS.

By H. A. EBERLE, M. D.,

GREENSBORO, S. C.,

CAPTAIN AND ASSISTANT SURGEON, UNITED STATES VOLUNTEERS.

The island of the Sulu, or Jolo, one of the Philippine group, lies between the sixth and seventh degrees north latitude; about seven hundred miles south of Manila. It is thirty-six miles long and about sixteen miles wide.

The inhabitants are principally Moros, over whom reigns a sultan. The chief occupation of these Moros is fishing and for that purpose they live near the water, mostly upon the water, and frequently under the water, especially when pearl fishing. They are considered to be a hardy race, owing, no doubt, to their simple mode of living almost outdoors, and to an exclusive diet of fish, fruit, and vegetables, as they abstain from stimulants and meats. From the best sources of information I have learned that these people are not prone to attacks from the almost prevailing endemic and sometimes epidemic dengue which sweeps the American colony.

The walled city of Jolo was built by Señor Arolas, the Spanish governor, whose frequent "scraps" with the Moros gave him many prisoners, and to this captain general and his Moro prisoners we are indebted for the most beautiful little city in all the Philippine archipelago.

But while the Spanish governor had an artistic brain in laying out a city with paved avenues, bordered on either side with fragrant tropical trees, the Y'llang-Y'llang predominating, with open and cemented sewers and many public and private gardens, with fountains, grottoes, and fragrant tropical plants, his honor, instead of choosing a beautiful plateau at the foot of a range of sublimely grand and picturesque mountains, with superb natural drainage, built his city principally upon the tidal mud flats. The governor may be excused if desire for his safety impelled him to encroach as little as possible upon the territory of the savage Moro.

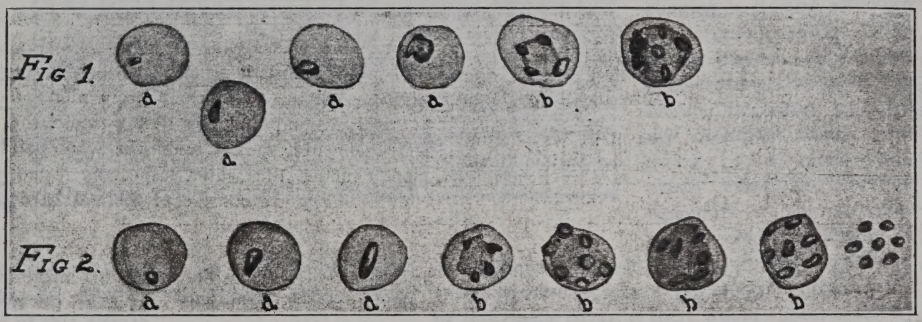
In consequence of his selection, the city is only from two to five feet above the sea level, according to ebb or flood tide. Naturally we should conclude that the location would be extremely unhealthful, but such is not the case generally, except during the summer and early autumn months, when dengue is most prevalent.

The prevalence of this disease in Jolo may be accounted for, first, in consequence of certain toxines which arise from the porous ground, used in cover-

ing the mud flats, on which are built the barracks and living quarters of the post, or, secondly, from one of the Culex variety of mosquitoes, *Culex fatigans,* which is found to propagate in the sulci of the cocoanut tree, and in every knothole capable of holding moisture. From one knothole containing about four ounces of water and débris, the writer developed over five hundred culices fatigantes. Upon examining this variety of mosquito, blood fed on acute dengue cases, the microscope revealed

was the peculiar type of fever, or temperature curve, differing from any of the malarial types. During the prodromal stage, which was short, there was pain in the loin and legs, with headache, the temperature gradually rising to 102° and 104° F., frequently not preceded by a chill; at most there might be chilliness. Often an attack of vertigo, or extreme dizziness, would usher in the disease.

During the drill hour or other fatigue duty, a soldier would fall out of the ranks, and probably



FIGS. 1 and 2.—*a, a, a, a,* Impregnated corpuscles; *b, b,* The growing parasite, in which early sporulation appears; *c,* Mature spores after escaping from the limiting membrane of the corpuscle.

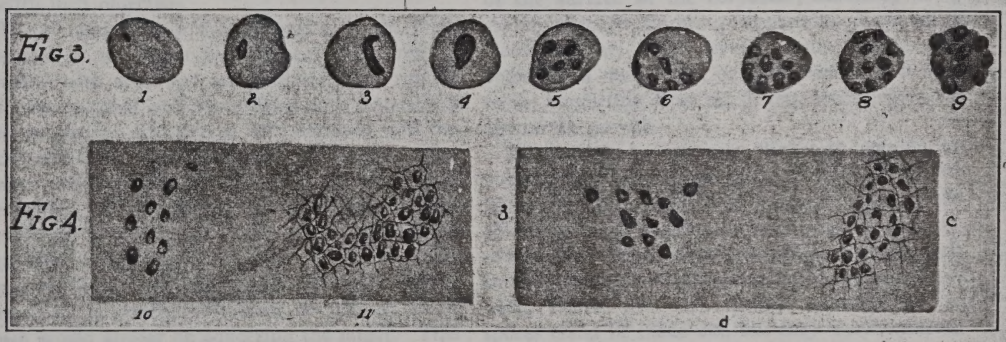


FIG. 4.—10, Mature parasites; 11, Phagocytes inclosing in their meshes myriads of dengue organisms.

the plasmœbic organism, and the writer suspects from these and other investigations, that culex fatigans is the transmitter of the disease.

During the summer months beginning about May 15, 1902, and ending about December 15th, an epidemic of dengue visited the city, attacking the soldiers and American residents principally.

The epidemic, however, spared neither race nor sex, for the Filipinos, Chinos, Malays, and Moros were afflicted to some extent, though less so than the Americans.

From what could be learned from the antecedent history of the natives and inhabitants of the islands, it appears that dengue is nearly always endemic.

Most noticeable during the course of the disease, fall to the ground from a severe attack of vertigo; on being brought into the hospital and a blood examination made, it would be found that the specific germ of dengue existed in the plasma.

By referring to the temperature charts, the peculiar fever curve can be seen. It approaches nearly a double tertian æstivoautumnal malarial fever of a remittent type. My attention was attracted to the peculiar rose colored rash which made its appearance from the fifth to the seventh day of the disease, and to the entire unresponsiveness of the fever to large dose of quinine.

The only medical literature upon the subject of dengue available at this post was the admirable work of Sir Patrick Manson, C. M. G., M. D., LL. D.,

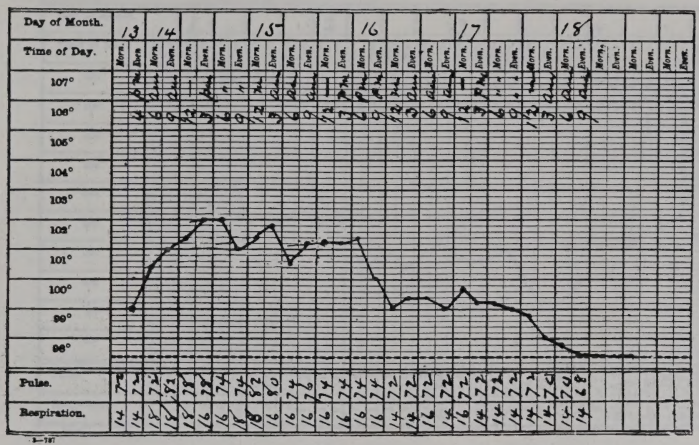


Chart No. 1.

Tropical Diseases, in which, on page 195, he states concerning the ætiology, "nothing is known as to the virus of this disease."

About June 1st the writer began to study fresh blood specimens through a Bausch and Lomb twelfth inch objective.

The minutest details of cleanliness were observed. The pad of the finger was chosen, and carefully cleansed with soap and water, dried, then rubbed with pure alcohol, and again dried with a sterilized piece of gauze. The needle was sterilized, and the slides and cover slips, previously washed in equal parts of ammonia and alcohol, then in pure alcohol, were dried with a soft silk handkerchief and protected with a glass cover. The first drop of blood was wiped away with a piece of sterilized gauze, dampened in alcohol, then dried, and a cover glass was ready to touch the minute droplet of blood which would appear on squeezing the finger. This was quickly placed on the glass slide, and sealed with Canada balsam or ringed with vaseline. Two to four slides would be thus prepared and made ready for examination. Very many observations were made and studied before the results were transferred in drawings to paper.

Comparisons were made between the blood of patients suffering from quotidian and tertian types of æstivoautumnal malarial fever, and that from healthy individuals. In many slides where the blood corpuscles were evenly spread out, small and almost imperceptible motile parasites could be seen dancing and gyrating in the plasma. It was noticed, especially when early examinations could be made, that this little motile parasite had the power of attaching itself to the border of a red corpuscle, apparently boring its way into it and clinging to it; and, although the corpuscles shifted freely in the plasma and bumped against other corpuscles, the minute organism would not disengage itself. An occasional motile body would enter the limiting membrane of the corpuscle and become confined in its enclosure, where it could be studied at pleasure. In the corpuscles, after being stung or impregnated by this motile parasite, there would appear a faintly tinted growth of a pale violet color, which in one or two hours would cover one fourth to one half of the surface of the corpuscle; assuming every variety of shape; throwing out pseudopodia in which would appear, after several hours, faint, dark bodies on a deep focus, or paler bodies on a light focus, numbering from six to ten, according to the age of the growth.

These dark bodies were the early sporulating bodies, which after aging would appear with definite outline and a pale bluish centre. These violet colored growths were motile and on further development became more active, propelling the corpuscle through the plasma and giving it the appearance of being full of life. No "daisy" arrangement of spores could be made out, but they were scattered

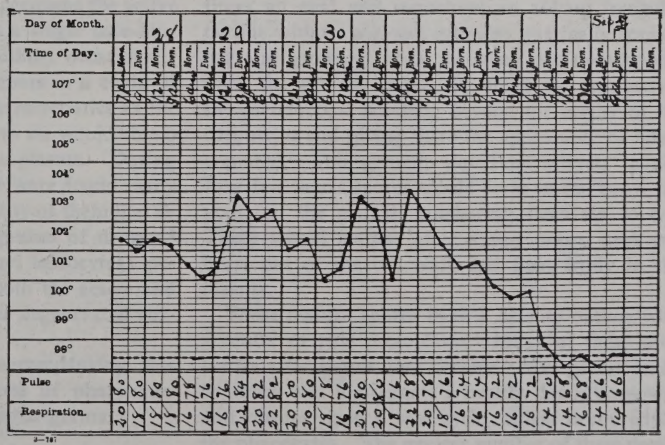
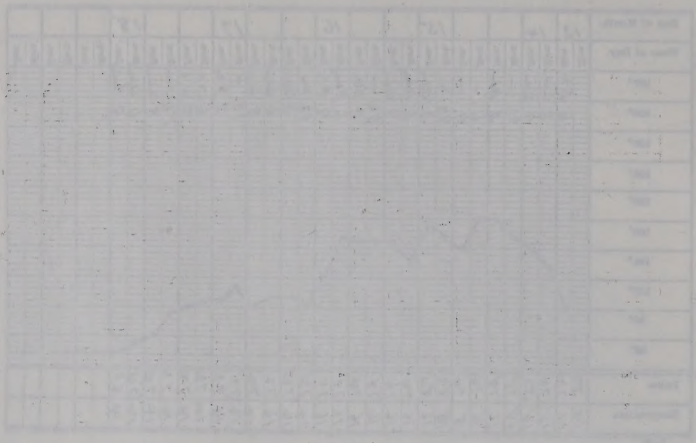
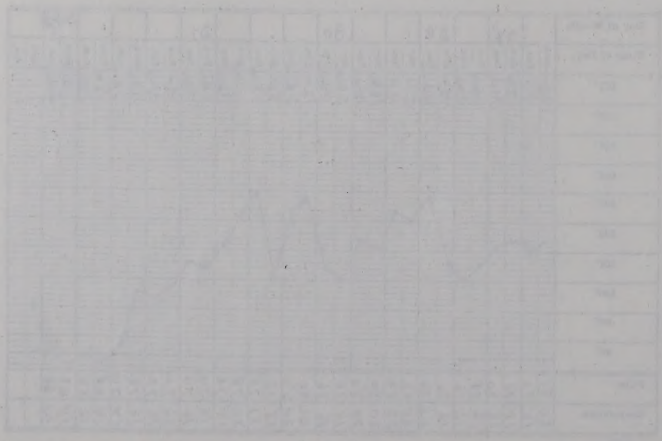


Chart No. 2.





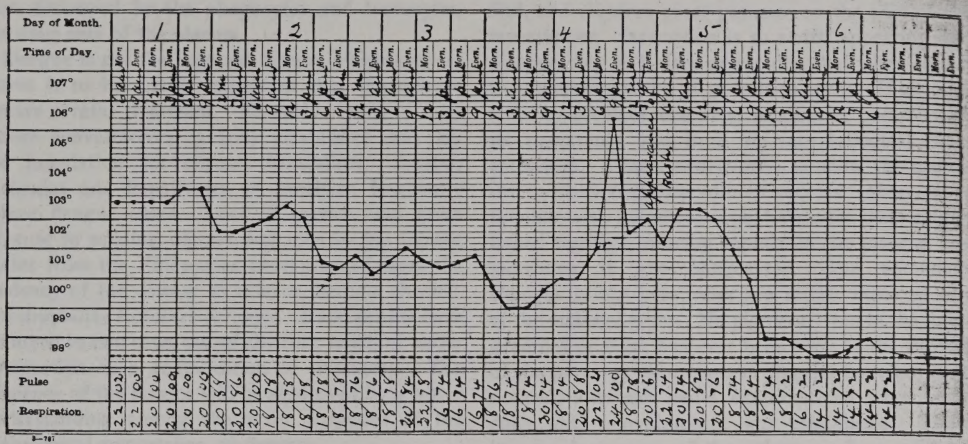


Chart No. 3.

irregularly like a plate of fruit over the surface of the corpuscles.

After being fully developed, these spores appeared not only to fill the corpuscles, but the outline of the corpuscles would become disintegrated and lose its contour, giving freedom to the enclosed spores. In all the specimens examined there could not be detected any pigment or melanin particles, as in tertian malarial fever. In no stage of the disease was there an absence of the active parasites from the plasma. They were most numerous, however, in the phagocytic stage, and were found especially in the meshes of those scavengers of the blood. As long as the high temperature lasted, which was usually from five to seven days before reaching normal, daily examinations would disclose the active parasite as well as the sporulating and segmenting bodies.

The cycle appeared to be less than twenty hours. Crescents were not discernible throughout the entire course of the disease. In many specimens the active parasite, instead of appearing as a single pale blue hyaline body with a definite outline, occasionally would be linked together with others in a chain of two or three, having the same characteristics as the single parasite. Sometimes four to six would clump together and move freely in the plasma. During the later stages of the fever there were found many phagocytes and leucocytes and many of them literally swarmed with the minute organism of dengue.[1] These scavengers (phagocytes and leucocytes) policed the plasma and destroyed both the active and passive parasites, as well as other degenerated corpuscles.

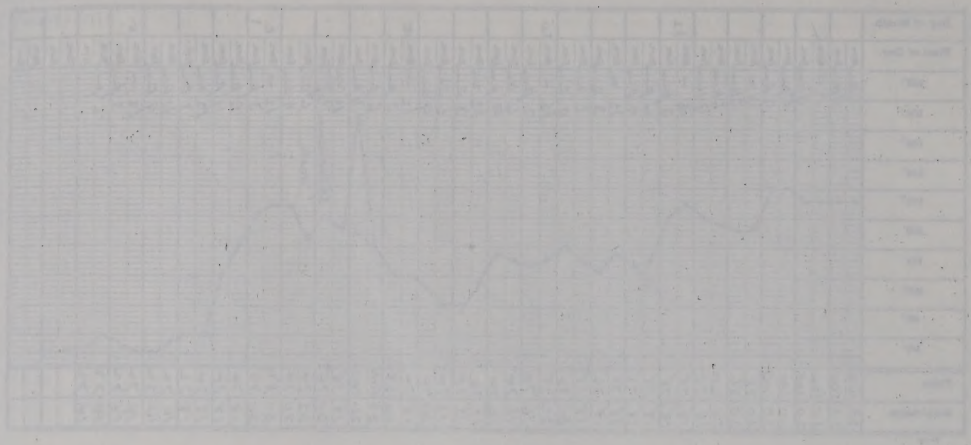
In the plates and drawings accompanying this report may be seen reproductions of phagocytes alive with myriads of motile dengue germs.

[1]See Charts 1 and 2.

In searching for these germs, the microscopist should take every precaution to observe absolute cleanliness in the preparation of his fresh blood specimens. If taken early in the morning the stage of the microscope should be warmed. A good plan to prevent crenation, is to prepare the slides and seal the cover slips with Canada balsam at once without exposure to the air; then lay them aside under a glass cover from two to four hours. The activity of the germs will be increased, and sporulation will proceed in the same manner as in subsequent fresh blood specimens. The active plasmœbæ have been noticed one hundred hours after sealing the slides in this way. The phases of the dengue parasite bear a close resemblance to the phases of the life cycle of the benign tertian parasite in man.

The secondary changes in the corpuscles, after becoming infected by the parasite, were shown in a pale growth or disc like body, assuming sometimes an oval and sometimes an oblong irregular outline without pigment granules, gradually spreading and growing, often throwing out pseudopodia until the growth completely filled the corpuscles; the pseudopodia reaching beyond the border of the corpuscles and giving them the appearance of being crenated. This growth would be actively motile. In a few hours spores would form in the body, the podia of the growth numbering from six to ten. These would mature after a few hours, segment, burst out of the cell wall, and float freely in the plasma.

These mature spores have a definite outline with a centre of pale protoplasm. Many of these little bodies after segmenting become active germs ready to attack and impregnate a healthy blood corpuscle and reenter upon a new cycle, unless devoured by the phagocytes. The greater number of these newly



fledged segmenting bodies are passive and are likewise destroyed by the phagocytes and leucocytes, the drag nets of the plasma.

Dengue is not the innocent disease which authors assert it to be. It is important that the disease receive careful treatment. An early return to duty before convalescence is fully established may lay the foundation for chronic invalidism. Serious cachexias and impairment of nutrition frequently follow dengue cases. Loss of service, and great expense in sending officers and men to the United States from the Philippines have resulted from the tendency of the disease to relapse, become chronic, and disqualify sufferers for duty. Fatal results have occurred in two cases out of the four hundred under observation as a complication of dysentery. The efficiency of the service will be subserved by a better understanding among the army surgeons of the pathological importance of dengue.

Frequently, after the active symptoms had subsided, the temperature had returned to normal, the tongue was clean, and the patient hungry, expressing a desire to return to duty, he would reenter the hospital with a slight headache and feverishness. During the next few hours a rose colored rash would appear on his face and neck, soon extending to his body, arms, and legs, accompanied by suffusion of the eyes. This eruption was rose colored, confluent, punctiform, often complicated with sudamina, and accompanied with slight itching. During the next two or three days the rash would slowly disappear by desquamation.

Histologically, the parasite of dengue properly belongs to the class Sporozoa, and since it is found first in the plasma of the blood, the writer has given it the generic name Plasmœba. This plasmœba, primarily found in the liquor sanguinis, impregnates the corpuscles, producing a pale, violet tinted growth assuming various shapes as it increases in size, and in which can be noticed, after two to four hours, faintly darker bodies, the earliest spore formations which occupy usually from two thirds of to the entire corpuscle. On many occasions, when the spores form in the pseudopodia, they give the corpuscles the appearance of being crenated, but in fact they are merely projections beyond the border of the corpuscle pushing out the elastic limiting membrane of the cell. These spores mature in the course of a few hours and are characterized by a definite outline inclosing a pale protoplasm without nucleus or nucleolus. These matured spores are highly refractive and finally burst through the limiting membrane of the corpuscle and are poured out into the plasma (the greater number, however, being passive) and ready to enter on a new cycle.

The dengue plasmœbic organism is smaller and much more active than the amœba in malarial blood. In fact, it is a most elusive and difficult germ to find, and requires great patience on the part of the investigator. Its motion is so swift, appearing and disappearing as it does so rapidly, that when shown to a microscopist he often fails to see it. If encountered by accident, one must familiarize oneself with its appearance and study its morphological characteristics. I have endeavored to show the organism to many surgeons without success; others, more familiar with the microscope, have seen them singly, linked together, moving rapidly in the plasma, and swarming in the phagocytes. The secondary changes in the corpuscles are unlike those of any of the malarial types. No pigment, even in the finest granules or dust, has ever been seen in the growth of the parasite. Crescents or flagella have not been noticed, although serial examinations were made daily until convalescence was established. There is no enlargement of the spleen or liver. Oligocythæmia and anæmia frequently occur, especially if the disease becomes chronic, which it sometimes does. The disease is sometimes associated with æstivoautumnal types, but the parasites are easily distinguished. The plasmœba can be found in fresh blood specimens during every day of the disease, and in the same specimens sporulation and segmentation go on continuously. This fact accounts for the peculiar temperature curve which is here described and is of diagnostic value.

THE TEMPERATURE CURVE OF DENGUE.

The fever never rises sharply. There is a gradual rise of fever, seldom preceded by chill or chilliness. After the initial fever has set in, the course is not marked by distinct remissions. There may be an unnoticeable and gradual rise of temperature, which frequently the patient thinks nothing about for two or three days, or there may be vertigo, dizziness, and nausea which send the patient into the hospital, when after investigation it is found that the patient has had a slight fever for several days. The writer has been called to see patients at the time of the appearance of the rash, but a history would reveal that fever and pain in the back, head, and legs had existed several days. When the height of the fever is reached by vacillating stages ranging from 102° to 104.5° F., attaining its fastigium in about forty-eight hours, there occurs a gradual vacillating remission of a degree or more during the early hours of the morning. During the middle and after part of the day a slight rise will be noticed, but the fever seldom attains the same height as at the end of the first forty-eight hours. The temperature goes along in this manner, declining each day until normal. There is seldom any sharp crisis throughout the entire course. It happened on one occasion that a critical rise to 106.2° F. occurred preceding the

appearance of the rash. This does not often happen. When uncomplicated cases occur, the temperature becomes normal on the sixth or seventh day.

Quinine has no effect in cutting short the disease, neither has it any effect in lowering the temperature. Only in complicated cases would its administration be recommended and then it should be combined with other antipyretics. Convalescence is established about the same time, with or without medicine.

The minute dengue organisms stain beautifully in weak eosin solutions and methylene blue. Without first being familiar with the histological and morphological characteristics of these bodies in fresh blood specimens, stained specimens would be of little help to the bacteriologist. The foregoing investigations were made during the months of June, July, August, and September, 1902.

Subsequent researches have confirmed the facts as set forth in my earlier reports to the surgeon general of the United States Army.